REMARKS

Applicant recognizes with appreciation that Examiner indicates that Claims 10 and 13 have been allowed.

In this Amendment, Applicant has amended Claims 14 – 15 and 33 – 34 to specify different embodiments of the present invention and overcome the rejection, and added new Claims 35 – 41. It is respectfully submitted that no new matter has been introduced by the amended claims. All claims are now present for examination and favorable reconsideration is respectfully requested in view of the preceding amendments and the following comments.

BACKGROUND:

Applicant believes that it will be helpful for the Examiner to better understand the present invention by referring to the following the background of the present invention.

Treatments that are currently used to prevent or attenuate (cardio)vascular-related disease events involve the use of anti-platelet agents and/or anticoagulants, which function by impairing platelet function and/or coagulation.

The most widely used anti-platelet agent [as an antithrombotic] is aspirin. Aspirin is used because it irreversibly inhibits the synthesis of platelet cyclooxygenase-derived thromboxane A_2 (TxA_2), a potent platelet agonist. The overall effect of aspirin, however, is modest and inhibition of TxA_2 only results in a benefit for some but not all patients.

The mechanism of action of the most widely used anticoagulant, heparin or one of its low molecular weight fractions (used as an antithrombotic), is to accelerate the inhibition of thrombin by antithrombin III.

The bleeding risks of both antiplatelet agents and anticoagulants are common general knowledge. Patients are asked to stop taking aspirin before most surgical

procedures because there is a risk of significant bleeding as a result of impaired haemostasis. Heparin and its low molecular weight fractions also are associated with significant bleeding side effects because the coagulation cascade is impaired.

Endogenous 13-HODE is known to be an important signal transduction molecule. Exogenous 13-HODE, in concentrations known to exist *in vivo*, has little or no effect on platelet or leukocyte function (as demonstrated by Haas *et al*, 1990; Bertomeu *et al*, 1990; Buchanan & Bastida, 1988; Buchanan & Brister, 1998; Buchanan *et al*, 1998).

The current difficulties associated with modulating <u>endogenous</u> vessel wall 13-HODE relate to a lack of known ingestible or injectable potent drugs to upregulate endogenous linoleic acid turnover and subsequent 13-HODE synthesis. Moreover, it is believed generally that purified 13-HODE (as with most known lipoxygenase-derived monohydroxides) is unstable and readily metabolized, like most signal molecules. Thus, the use of exogenously-added 13-HODE was considered to be an unlikely option to upregulate endogenous vessel wall 13-HODE.

Most studies assessing 13-HODE have focused their attention on the potential short term effects of exogenous 13-HODE on assorted biological pathways (e.g., pages 10-11). In contrast, Applicant has surprisingly discovered that 13-HODE given orally in doses >1000 fold less than doses suggested by most other studies, effectively: a) enhances endogenous vessel wall 13-HODE level, an effect that is sustained (Figure 2); b) attenuates the onset of vessel wall hyperplasia (Figures 3 & 5); which, in turn, c) facilitates long term vessel wall remodeling and hyperplasia regression (Figure 3); and these effects are achieved at very low doses that are measured in ng/ml amounts in circulating plasma (Figures 1 & 4). Investigators such as Streber and Kaminakai, while they proposed different mechanisms of action using >100-fold higher doses of fatty acids; (i.e., in non-physiological concentrations including 13-HODE), have failed to demonstrate any potential benefit *in vivo* in any pathological state (pages 10-11).

The current invention discloses the use of 13-HODE administered orally in surprisingly low doses, to increase the endogenous levels of 13-HODE in vessel walls, thus rendering them less thrombogenic (i.e. biocompatible with circulating blood cells).

CLAIMS OBJECTIONS:

Claim 33 has been objected as containing certain informalities.

It is respectfully submitted that the objection has been overcome by the currently resented amendment. More specifically, Claim 33 has been amended to correct the improper Markush-type language by replacing "or" with "and." Therefore, the objection has been overcome. Accordingly, withdrawal of the objection is respectfully requested.

REJECTIONS UNDER 35 U.S.C. § 112 FIRST PARAPGRAPH:

Claims 15 and 34 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

It is respectfully submitted that the rejection has been overcome by the currently resented amendment. More specifically, Claims 15 and 34 have been amended to replace "at least one" with "a" to clearly define the embodiments of the invention. Moreover, Claim 41 has been added to specify additional embodiments of the invention.

In addition, the Examiner recognizes that the instant specification discloses a composition comprising 13-HODE in its free form or with a pharmaceutically acceptable carrier (Office Action, page 3, lines 13 – 14). Furthermore, the Examiner recognizes that "the specification provides sufficient written description for the composition **comprising** (A) 13-HODE ... and (C) carrier ..." (Office Action, page 4, lines 8 – 15 from bottom, emphasis added). Therefore, there is adequate written description in the specification with regard to the embodiments defined in Claims 15, 34 and 41.

Therefore, the rejection under 35 U.S.C. § 112, first paragraph has been overcome. Accordingly, withdrawal of the rejections under 35 U.S.C. § 112, first paragraph, is respectfully requested.

REJECTIONS UNDER 35 U.S.C. § 102:

Claims 15, 17 and 34 have been rejected under 35 U.S.C. § 102 (b) as allegedly being anticipated by Streber (US 5,102,912), hereinafter Streber.

Applicant traverses the rejection and respectfully submits that the present-claimed invention is not anticipated by the cited reference. More specifically, Streber fails to disclose, explicitly or inherently, all the limitations of Claims 15, 17 and 34. In addition, Claims 15 and 34 have been amended to further specify that "for use in increasing the endogenous levels of 13-HODE in vessel walls in a subject." Claim 17 also includes this feature due to their dependency on Claim 15.

At first, Streber refers to the use of 13-HODE in excessively high, non-physiological doses for the treatment of tumour growth and targets the aromatase-dependent modulation of estrogen-androgen hormone regulation. As discussed below, Streber: a) proposes doses of 9-HODE and 13-HODE 40- to 100-fold higher than the dose of 13-HODE proposed in the current invention; b) teaches that 9-HODE is more effective than 13-HODE; c) focuses on tumour growth and not vessel wall biocompatibility; and d) focuses on aromatase enzyme inhibition by exogenous 9-HODE and not vessel wall integrin expression regulation by endogenous 13-HODE.

Streber's patent focuses on the proposed use of exogenous 13-HODE to inhibit aromatase enzymes involved in the modulation of estrogen and androgen hormone regulation, the dysfunction of which can lead to breast carcinoma and prostrate hyperplasia. Streber's rationale is based upon the observations that: a) aromatase inhibitors attenuate breast carcinomas and benign prostate hyperplasia; b) a 10 minute exposure of aromatase to 1-100 mg/L of 9-HODE or 13-HODE inhibits aromatase activity, using placenta microsomes (i.e. not in intact cells and not *in vivo*). Streber provides no evidence that ingested 13-HODE, in any form, is duly absorbed across the gastrointestinal barrier, remains stable, targets uterine prostate or placental cells/tissue or exerts any effect on estrogen-androgen regulation. Streber only speculates, therefore, that a daily dose of 100-1000 mg, preferably 200-500 mg daily, is beneficial.

Streber proposes to target the aromatase enzyme involved in estrogen-androgen regulation and tumour growth. Streber teaches that one would need a dose of 200 to 500 mg daily, which is 40- to 100-fold higher than the dose demonstrated in the present invention, to be effective in preventing vessel wall hyperplasia *in vivo*. Moreover, the observations that both 9-HODE and 13-HODE in a concentration of 1 mg/l had ≈ a 45 % inhibitory effect on aromatase activity and 9-HODE in a dose of 100 mg/l had ≈ a 94 % inhibitory effect that was 4-fold more effective than 13-HODE in the same concentration (Table 4, column 6) would teach that a) 9-HODE was more effective than 13-HODE, and b) one must increase and not decrease the concentration or dose in order to achieve a desirable effect which is, in this case, the inhibition of aromatase activity, and perhaps tumour growth. Finally, it should be noted that, to date, this treatment for breast carcinomas or benign prostrate hyperplasia has not been shown to be of any benefit.

Streber teaches that 9-HODE is more effective than 13-HODE. A person of ordinary skill in the art would know that vessel wall cells only metabolize 13-HODE from linoleic acid via the lipoxygenase pathway, and that 9-HODE synthesis is only an epi phenomenon associated with cyclooxygenase-dependent metabolism of excessive, non-physiological amounts of exogenous linoleic acid (Buchanan *et al.*, J. Biol. Chem. (1985) 260:16056-16059; Buchanan *et al.*, Iscosanoids in Blood and Vascular Cells (1987) 152:247-258).

Streber proposes giving industrial (i.e. non-physiological) amounts of 13-HODE to act as an antagonist of aromatase activity to mediate an anti-tumour effect. Streber demonstrates that there is a significant concentration-related response but teaches <u>away</u> from the use of a low of 13-HODE in the attenuation of aromatase activity. As noted above, the present application demonstrates that a dose of 13-HODE 40- to 100-fold less than that suggested by Streber is effective, albeit for the treatment of a different disease and by a different mechanism of action.

Furthermore, Streber proposes to use 13-HODE as an antagonist, totally unrelated to the re-establishment of homeostasis as is any treatment used to re-establish physiological well-being (e.g. the use of insulin in diabetics for glucose metabolism, the use of Factor VIII in haemophiliacs for normal coagulation or, as in the present invention, the use of 13-HODE in patients with vascular dysfunction for biocompatible vessel

walls). Streber teaches the impairment of a normal biological pathway because another pathway is dysfunctional.

Therefore, the embodiments of the present invention as claimed are different from Streber, and they have components and effects which are not disclosed nor taught in Streber. In summary, the newly presented claims are not anticipated by Streber and the rejection under 35 U.S.C. § 102 (b) has been overcome. Accordingly, withdrawal of the rejections under 35 U.S.C. § 102 (b) is respectfully requested.

REJECTIONS UNDER 35 U.S.C. §103:

Claims 14 – 17, 19 – 23 and 33 – 34 have been rejected under 35 U.S.C. §103 as allegedly being unpatentable over by Vanderhoek et al. (US 6,077,525), hereinafter Vanderhoek, in view of Breivik et al. (US 5,502,077), hereinafter Breivik. Claim 18 has been rejected under 35 U.S.C. §103 as allegedly being unpatentable over Streber in view of Carlsson et al. (WO 99/44585), hereinafter Carlsson.

Applicant traverses the rejection and respectfully submits that the embodiments of present-claimed invention are not obvious over Vanderhoek in view of Breivik. At first, Claims 14, 15, 33 and 34 have been amended to further specify that "for use in increasing the endogenous levels of 13-HODE in vessel walls in a subject." Claims 16 and 19 – 23 also include this feature due to their dependency on Claims 14 – 15.

It is respectfully submitted that Vanderheok teaches that exogenous 13-HODE inhibits platelet TxA_2 synthesis and increases platelet 12-HETE synthesis (Setty *et al*, EMBASE abstract AN 87222961 (1987)). It is widely known that increasing platelet 12-HETE synthesis increases platelet adhesivity and prothrombotic potential (for example: Buchanan *et al*, Prostaglandins Med 1986; 21: 157-168; Buchanan *et al*, Am Heart J 1987; 113; 1133-1137; Van-Ryn & Buchanan, Prost Leuko Essential Fatty Acids 1989; 36: 171-174; Buchanan & Brister, Can J Cardiol 1995; 11: 221-227; Buchanan & Bastida, Med Hypothesis 1988; 27: 317-325; Buchanan & Brister, Adv Exp Med Biol

1997; 433: 265-269; Cortolazzo *et al*, Am Heart J 1998, 57: 277-282; Buchanan *et al*, Prost leuko Essential Fatty Acids 1998; 58: 339-346).

Thus, a person of ordinary skill in the art would question the rationale of using any intervention therapy that increases platelet 12-HETE synthesis. While Vanderhoek may teach the concept that CLA > 13-HODE > 9-HODE inhibits platelet function, it only demonstrates that CLA >13-HODE > 9-HODE inhibits a) platelet-derived TxA_2 (measured as TxB_2, sheets 2 & 3); b) arachidonic acid-induced platelet aggregation (sheets 4 & 5); and c) calcium ionophore-, collagen- and thrombin-induced platelet aggregation in a concentration-related manner (sheet 5), all of these effects allegedly achieved without affecting 12-HETE synthesis. However, anyone with ordinary knowledge about platelet function and fatty acid metabolism would know that TxA_2 synthesis occurs at the outer platelet membrane surface via the cyclooxygenase pathway utilizing molecular oxygen, and reaches peak amounts within 30 to 120 seconds, whereas 12-HETE synthesis occurs intracellularly via the lipoxygenase pathway, and reaches peak levels within 30 minutes (Lagarde M *et al*, Biochem J (1984) 222:495-500; Lagarde M et al Prog lipid Res (1988) 27:135-152; Lagarde M *et al* Adv Exp Med Biol (1999) 447:87-93; Markus AJ *et al*, J Biol Chem (1988) 263:2223-2229; Buchanan *et al*, Biol Icosanoids in Blood and Vascular Cells (1987) 152:247-258).

Moreover, 12-HETE is <u>not</u> released from the platelet unless the platelet membrane is disrupted (see above references). Thus, a person of ordinary skill in the art would not come to the conclusions suggested by the Examiner. Specifically, if one looks critically at the chromatograph shown in Figure 1 (sheet 2), it is clearly apparent that 12-HETE production (within the 2 minute time frame of the experiments) is higher in lanes 5, 6 and 7 than in lanes 4 which is higher than in lanes 2 and 3, which are comparable to lane 1. Applicant submits that Vanderhoek provides <u>no</u> evidence that CLA, 9-HODE or 13-HODE does not have any effect on 12-HETE synthesis and, in fact, provides evidence that these fatty acids (in the concentration used in this patent) actually enhance 12-HETE synthesis. Consequently, according to Vanderhoek, while platelet aggregation may be impaired, platelet adhesion and thrombotic events may be potentiated. This paradoxical hemorrhagic/thrombotic effect has been demonstrated clinically both in patients with essential thrombocythemia (Cortolazzo *et al.*, Am Heart J (1998) 57:277-282) and in

patients undergoing coronary bypass grafting (Buchanan & Brister, Can J Cardiol (1995) 11:221-227).

Vanderhoek teaches a daily oral dose of 0.25 to 0.5 g/kg (column 4, lines 6 to 8). Such a dose translates into a 17.5 to 35 g daily dose for a 70 kg patient – at least 5-fold higher than any fatty acid therapy dose used in the past, used currently or recommended for use thus far. Vanderhoek's dose, translated into a 250,000 to 500,000 μg/kg body weight dose, is at least 25,000-fold higher than proposed by the present application.

Vanderhoek refers to the use of conjugated fatty acids, seconded by 9-HODE and by 13-HODE respectively, to inhibit platelet-derived TxA_2, while allegedly sparing an effect on platelet-derived 12-HETE. The applicant argues, however, that the approach followed by Vanderhoek would <u>exacerbate</u> 12-HETE-dependent platelet adhesion and subsequent thrombus formation, and not attenuate it.

Breivik teaches that omega-3 fatty acids may reduce mild hypertension, reduce plasma cholesterol levels, and/or inhibit platelet function (column 1, lines 37-40). The mechanism of action of the n-3 fatty acids appears to be associated with their ability to compromise the synthesis of the platelet-derived arachidonic acid prostanoid, TxA_2, a potent platelet agonist and vasoconstrictor. The dose of these n-3 fatty acids needed to achieve such an effect in vivo is 3 gm/day of total fatty acids comprised of >80% active omega n-3 substrates (column 1, line 6; column 6, Table 3). Breivik further teaches that the use of the omega n-3 fatty acids to attenuate hypertension, can reduce diastolic (column 7, Table 4) and systolic blood pressure (column 8, Table 6), whereas other fatty acids, such as corn oil (rich in linoleic acid, the substrate for 13-HODE), have no effect. Moreover, Breivik teaches that the composition of the potent omega n-3 test materials contain little or no linoleic acid (i.e., < 0.3% 18-2, n-6, column 5, Tables 1-3).

A person with ordinary skill in the art would interpret Breivik as teaching that omega-3 fatty acids, in a dose that is 49,800-fold higher than the dose identified in the present application, may be useful in reducing mild hypertension, presumably associated with TxA_2-induced vaso-constriction, although no evidence or direction is provided in this regard. A person skilled in the art would also be aware that two major side effects associated with high dose fish oil intake are flatulence and noxious body odour, both of

which have lead to poor patient compliance and that omega-3 fatty acids do not provide any real clinical benefit to patients with coronary artery disease with or without mild hypertension and/or hyperlipidemia (Cairns JN *et al.*, The EMPAR study, Circulation (1996) 94:1553-1557).

In contrast, a person skilled in the art would be aware that the classes of drugs known as statins (lipid lowering drugs) and as ACE inhibitors and diuretics (antihypertensive drugs) have been shown to be far more effective [in the treatment of coronary artery disease]. A person of ordinary skill in the art would not infer or deduce from the above observations, either directly or indirectly, that very low dose 13-HODE would play any role in achieving the effects alleged above since the lower concentrations of the fatty acids above were less efficacious that the higher concentrations, and for the most part contained little or no linoleic acid or 13-HODE.

Breivik refers to the use of marine fish oils in the treatment of mild hypertension related to TxA_2-induced vasoconstriction and a reduction in total serum cholesterol. A person skilled in the art is aware that malignant hypertension is multi-factorial, idiopathic or associated with renal dysfunction and not due to TxA2-induced vasoconstriction, which is a secondary consequence of platelet activation associated with normal hemostasis. Breivik also excludes linoleic acid in the preparation of the omega-3 supplements and does not discuss 13-HODE.

The Examiner states that the teachings of Vanerhoek and Breivik make clear that the use of 13-HODE for inhibiting platelet aggregation or lowering LDL-cholesterol are well known in the art. The Applicant respectfully disagrees. A person of ordinary skill in the art would be aware that Lee *et al.* (Atherosclerosis (1994) 108:19-25) reported that total and LDL-cholesterol were lower, although not significantly, in 12 rabbits fed a 14% fat + 0.1% cholesterol diet supplemented with 200 mg/kg daily of conjugated linoleic acid (CLA). In a subsequent study in mice, the same authors reported that a possible mechanism of action of such a treatment may be related to the ability of high dose CLA to suppress the expression of the stearoyl-CoA desaturase enzyme gene (Lee *et al* Biochem Biophys Res Commun (1998) 248:817-821), thus confirming observations by others performed 5 years earlier (DeWille JM, Farmer SJ, Biochim Biophys Acta (1993) 1170:291-295).

A person of ordinary skill in the art would also know that assorted LDL-receptor blockers, such as the statins, were shown to be immensely efficacious. The use of lipid lowering drugs such as the statins has resulted in a >22% reduction in morbidity and mortality in CVD patients over the last 15 years. A person of ordinary skill in the art would not think it obvious to pursue the use of CLAs as a treatment for hyperlipidemia, particularly when the only support in that regard are data generated in a few rabbits and mice fed an abnormally high fat content diet and treated with an excessively high dose of CLA. It should also be noted that Lee *et al* concluded that the inhibitory effect on CoA desaturase was independent of the monohydroxide isomers.

A person of ordinary skill in the art would not be led directly or indirectly to the use of 13-HODE, in a 1000-fold lower dose, to alter vessel wall adhesivity based upon the teachings of Vanderhoek or Breivik, who used different fatty combinations at excessively higher doses to affect platelet function, and/or to inhibit the CoA desaturase gene expression when statins are effective in reducing hyperlipidemia.

Furthermore, a person of ordinary skill in the art would not be led by Vanderhoek and Breivik to conceptualize a different mechanism of action to achieve a beneficial effect in patients, using a 40- to 25,000-fold lower dose of specifically purified 13-HODE.

Regarding the rejection based on Claim 8, Carlsson teaches that oil-in water emulsions containing evening primrose oil and 13-HODE are effective skin moisturizers when applied topically. The Examiner cites Carlsson as evidence that evening primrose oil (and other essential fatty acids) are suitable 13-HODE carriers.

It is well known that topical applications of evening primrose oil-in-water emulsions attenuate atopic dermatitis and epidermal cell hyperplasia (for example: Gimenez-Arnau A *et al.*, Adv Exp Med Biol (1997) 433:285-289; Cho Y, Ziboh VA, Biochem, Biophys Res Commun (1994) 201:257-265; Ziboh VA, Adv Exp Med Biol (1997) 433:279-283). These references demonstrate that linoleic acid, the substrate for 13-HODE which comprises 8-15% of the evening primrose composition, is incorporated into epithelial cells, providing increased amounts of the substrate for 13-HODE synthesis.

Gimenez *et al.* confirmed that atopic dermatitis is an essential fatty acid disorder, in particular related to a linoleic acid deficiency and both Ziboh and Gimenez teach that endogenous 13-HODE acts intracellularly in epithelial cells to achieve its beneficial effect. A person of ordinary skill in the art would readily recognize that the beneficial effect is not due to 13-HODE added extracellularly, but rather due to the epithelial cells preferentially incorporating linoleic acid, thus reversing the fatty acid deficiency defect. The intracellular linoleic acid, which is stored in the intracellular triglyceride stores (Haas *et al*, Biochem Biophys Acta (1990) 1031:174-178), is readily available as a substrate for intracellular 13-HODE synthesis. Intracellular 13-HODE, in turn, co-localizes with the lipophilic site of integrins spanning the cell membrane phospholipid bilayer (Buchanan MR *et al.*, Blood (1993) 81:3303-3312).

Carlsson speaks to 13-HODE as a topical cream for dry skin associated with exposure to detergents. Carlsson alludes to the instability of 13-HODE when it is pointed out that the 13-HODE, in 13-hydroxy-linoleic acid form, was added after emulsification, homogenization and cooling. Thus, a person with ordinary skill in the art would recognize the oxidative potential of that monohydroxide form, the selective uptake by epithelial cells of linoleic acid and its metabolism to intracellular 13-HODE.

Carlsson addresses the issue of using oil-in-water emulsion containing high concentrations of evening primrose oil and 13-HODE to be applied topically to attenuate dry skin irritation associated with the frequent use of detergents. The present application addresses the issue of using purified 13-HODE administered orally to be selectively incorporated by vessel wall cells to regulate vessel wall/blood cell interactions.

The Applicant respectfully submits that there are no obvious relationships between any of: a) the mechanisms of action; b) the biochemical pathways; and c) the target cells and tissues purported in the cited references and those identified in the current patent application.

Furthermore, the doses proposed in the cited references are in significant excess to the doses proposed in the current application. There is no evidence in the references to suggest that an oral ingestion of a low daily dose of 13-HODE (100 mg/kg) would have any effect.

Therefore, the rejection under 35 U.S.C. §103 has been overcome. Accordingly, withdrawal of the rejections under 35 U.S.C. §103 is respectfully requested.

Having overcome all outstanding grounds of rejection, the application is now in condition for allowance, and prompt action toward that end is respectfully solicited.

Respectfully submitted,

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